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C. I.

BACTERIAL ANALYSIS OF GROUND BEEF
WITH SPECIAL REFERENCE TO
SANITARY STANDARDS.

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A. INTRODUCTION

Meat has been judged, largely, for market use upon its physical appearance. This has not given the sanitarian a means by which he can predict the keeping qualities or the toxic effects of the food. Two laboratory methods have been offered as a basis for a possible standard for analysis,—total bacterial count and the ammoniacal* test. Little of practical value has been made available in either case because of the fact that so few examinations have been carried out. Stimulated by the work of Kühne, Mace and others on the relation of aerobic bacteria to putrefaction, Tissier and Martelly 1902 (28) worked on putrefaction in meat. In their work they isolated and described nine aerobic bacteria (four cocci and five bacilli) and four anaerobes. After analyzing the chemical reactions of these organisms, they concluded that putrefaction was due to the successive action of mixtures of these species. They concluded that this took place in the three steps: the 1st due to action of the aerobic species; the 2nd to anaerobes; and the 3rd to a mixture of the two.

* Ammoniacal test: Ebers' test (6). Presence of ammonia detected by means of HCl. Hydrochloric acid in the presence of ammonia combine to form a gray to white vapor.

Marxer 1903 (19) made a study of Hamburger steak and offered as a possible standard 1,000,000 bacteria per gram or the presence of a large number of the representatives of the "protein group."

In 1914 Weinzirl and Newton (32) studied samples of Hamburger steak in Seattle, Washington. They compared the bacterial count with organoleptic and ammoniacal tests. They concluded the Marxer standard was too low and made a standard of 10,000,000 bacteria per gram, thereby condemning 50% of their samples. There was an agreement between the organoleptic tests and ammoniacal tests but not between these and the count. When the ammoniacal test responded, the meat was so contaminated with bacteria that it was literally putrid. They suggested that 50% of all Hamburger steak should be condemned on general principles.

Carey 1916 (4) worked on the bacteriology of sausage in Chicago. This, according to Tanner (27), was essentially the bacteriology of Hamburger steak. From 34 samples he isolated *Bacillus coli* 30 times and *Bacillus proteus vulgaris* 11 times. He concluded that the bacterial count was of little significance. Cooking destroyed a large number of the bacteria present.

In 1917 Le Fevre (16), using Weinzirl and Newton's methods and standards, studied 22 samples of Hamburger steak from the Washington, D.C. markets. His counts varied from 35,000 to 35,000,000 bacteria per gram on plates read from three to five days after plating. The number of liquefiers present did not correlate with the organoleptic test or sanitary conditions. Of the bacilli, *Bacillus coli*, *Bacillus proteus vulgaris*, and some spore formers were isolated. He also reported cocci. Only in one instance was an anaerobe identified, although all samples gave a putrid odor when grown anaerobically for 24-48 hours. He found cooking for ten minutes on a hot plate did not sterilize the meat.

By far the most exhaustive and the best work on decomposing foods was that of Hunter 1920-22 (10-13) in his study on "feedy salmon." He thought a count of 15,000,000 was enough to explain decomposition of fish. He isolated the same aerobic organisms from the water in which the fish was found as he did from the decomposing muscle.

B. METHODS USED

1. Sanitary Rating of Shops

The purpose of this investigation was to determine (1) the relation of the bacterial flora of meat to the sanitary surroundings; (2) what factors affected a possible change in this flora; (3) what would constitute a possible bacterial standard of meat analysis. No chemical work was done and no attempt was made to explain chemically the steps in putrefaction.*

The material used was ground beef purchased in the local markets. This was chosen because of the fact that it offered (1) a very large bacterial flora; (2) a fruitful field for the study of the environmental conditions and bacteria present. The sanitary conditions of the place of purchase and the method of handling and wrapping the meat were carefully noted and graded as is indicated in Table I.

*Putrefaction as used in this paper is defined as the action of microorganisms on nitrogenous material with the production of a foul odor.

TABLE I

Sanitary Rating of Shops

(1) Basis Used for Grading Shops

1. Cleanliness of place of purchase.....	30%
2. Cleanliness of person handling meat.....	30%
3. Counter conditions { covered	20%
{ uncovered	
4. Method of handling meat { hands	20%
{ paddles	
{ paper	
Total	100%

(2) Clean Shop: 1 A

1. Counter: white granite clean; no odor of foul meat.....	30%
2. Clerk: neat, clean appearance; hands, coat clean	25%
3. Covered counter; meat in clean granite pan	20%
4. Meat handled with clean paddles, weighed on paper	20%
Total	95%

(3) Dirty Shop: 3 B

1. Shop: dark, dirty, wooden counter; foul odor throughout place ...	5%
2. Clerk: filthy coat and dirty hands.....	2%
3. Meat in dirty granite pan in ice box.....	10%
4. Meat handled; weighed with paper in small dish	15%
Total	37%

It must be remembered that the shop conditions are only one contributing factor to the bacteria present. The use of the term sanitary condition is applied here only to the condition of floor, counters, and ice boxes, as observed in the place of purchase. The source of the meat and its history before reaching the market was not known.

2. Organoleptic* Rating of Meat

On reaching the laboratory the meat was graded with the organoleptic test and was divided into groups, as is shown in Table II. Four points were used as a basis of these grades. The first was the quality of the meat as indicated in the good samples by the relatively low proportion of fat to the high proportion of muscle and in poor samples by the reverse conditions. The second was the presence of such foreign material as hair and vegetable matter, which frequently was found. The color was the third point. Any deviation from the normal appearance of the muscle was recorded. Odor, the last consideration, was found absent in some samples, while in others there was a distinct stale or strong smell but it was not foul.

*Organoleptic test describes odor, color and taste of meat as observed through the senses of sight, taste and smell.

TABLE II.

Organoleptic Rating of Samples

- | | |
|-------------------------|---|
| 1. Excellent-Very Good: | Color normal; no odor; small amount of fat; no foreign material present. |
| 2. Good: | Color normal; no odor; medium amount of fat. No foreign material present. |
| 3. Medium-Fair: | Slight discoloration; odor; large amount of fat; no foreign material present. |
| 4. Poor: | Discolored; strong odor; large amount of fat. Much foreign material present. |

3. Method of Making Aerobic Examination of Samples

The samples were placed in sterile covered glass jars in the ice box and observations were made at the end of each day to note change in color and odor. It was found that two day intervals were enough to show the earliest marked definite changes. The latter were marked by the appearance of a change in color, the presence of a strong or sweetish odor, and in some cases the growth of mold. Therefore, this was chosen for the period of bacterial examinations.

Two methods have been offered in the literature for the examination of meat. Weinzirl and Newton's (32) method was that of grinding meat in a mortar with sterile sand, washing out with 100 cc. sterile water

and plating on ordinary media. In the other, St. John's method, as cited by Tanner (27), the meat was shaken with sterile glass beads in sterile water and plated in ordinary media. Both of the methods were tried. The second was modified by using the mechanical shaker. Counts in four out of five samples showed little variation in 24 hour plate readings in the two. The latter was adopted because larger quantities of meat could be handled.

Twenty-five grams of meat were used for each examination. This was placed in a sterile glass quart jar. Sterile water was added to bring the dilution to 1 - 20. In this sterile water and meat, glass beads were placed and the jar was shaken for ten minutes on a mechanical shaker. From this emulsion, dilutions of 1 - 200 and 1 - 2000 were made, while in some cases it was necessary to make a third dilution, 1 - 20,000. Two plates of 1 cc. of each dilution were made with 2% beef infusion agar. In the latter part of the work dextrose litmus agar was employed to determine the presence of dextrose fermenting organisms. This was chosen in preference to lactose litmus agar in order that the presence of microorganisms which do not ferment lactose, such as *Bacillus proteus vulgaris*, might be readily noted.

At first the plates were incubated in duplicates at 1.2° C., 29° C., 37.5° C. The latter was found to show a representative count and was adopted. The averages of the four plates, read after 24 hours' incubation, were used as a basis to determine the count. All colonies appearing on the plates were transferred to agar slants for future type study.

C. COMPARATIVE STUDY OF BACTERIAL COUNT,
ORGANOLEPTIC TEST, AND
SANITARY CONDITIONS.

1. Relation of Count to Sanitary Conditions
of Place of Purchase

The meat was purchased at 49 places of extreme sanitary conditions; 22 samples came from public markets and 27 from shops.* Of the former two types of markets were chosen,—that in which the meat stalls were under shed with both ends open and that in which the shed[†] was practically closed. In the open shed eight samples were obtained from stands at both ends and the middle of the market.

*Shops are places of purchase not in public markets.

†Shed is a common covering of a number of stalls in a public market.

TABLE III

Market Samples Not Ground for Purchaser

Samples	Sanitary Rating of Shop	Organoleptic Test	Bacteria per gram	Dextrose Fermenters per gram
10 C	80%	Good	5,000	0
2 B	95%	"	16,000	2,000
29 C	80%	"	86,000	0
31 C	70%	"	96,666	0
7 A	70%	"	115,000	12,000
32 C	85%	"	173,000	4,000
5 A	65%	"	177,000	2,000
1 A	95%	"	296,500	0
3 C	50%	Medium	20,000	0
* 24 C	70%	"	31,000	2,000
33 C	70%	"	72,000	0
* 1 B	70%	"	169,000	10,000
* 4 C	65%	"	933,000	0
30 C	50%	Fair	42,666	2,000
22 C	50%	"	136,000	2,000
* 2 A	55%	"	2,075,000	6,000
* 4 A	37%	"	2,800,000	22,000
6 A	40%	Poor	4,800,000	26,000
* 3 A	75%	"	10,000,000	0

Market Samples Ground for Purchaser

* 2 C	80%	Very good	220,000	-
* 1 C	80%	" "	408,000	-
25 C	80%	Good	98,000	40,000

* Exposed to street dust.

TABLE IV
Shop Samples Ground for Purchaser

Samples	Sanitary Rating of Shop	Organoleptic Test	Bacteria per gram	Dextrose Fermenters per gram
9 C	90%	Very good	6,000	2,000
*13 C	95%	" "	7,333	4,666
16 C	90%	" "	21,000	2,000
6 C	90%	" "	34,000	2,000
14 C	90%	Good	6,000	2,000
28 C	75%	"	7,000	0
†11 C	50%	"	17,000	15,000
†21 C	90%	"	19,333	12,000
18 C	70%	"	32,000	0
*17 C	70%	"	37,000	2,000
5 C	55%	"	41,000	3,000
†12 C	70%	"	300,000	1,000
19 C	80%	Medium	18,000	2,000
26 C	30%	"	18,000	23,000
†15 C	30%	Fair	11,000	0
23 C	30%	"	13,000	8,000
*20 C	70%	"	27,000	5,000
27 C	30%	Poor	39,000	6,000

*Grinder cleaned.

†Old grindings put into meat.

‡Cleaver cut

TABLE V

Shop Samples Not Ground for Purchaser

Samples	Sanitary Rating of Shop	Organoleptic Test	Bacteria per gram	Dextrose Fermenters per gram
8 C	70%	Medium	8,600	2,000
*7 B	70%	"	64,500	8,000
34 C	70%	"	93,000	2,000
*7 C	70%	"	154,000	11,000
*6 B	85%	"	490,000	28,000
*5 B	32%	"	550,000	2,000
4 B	85%	"	1,424,000	26,000
3 B	32%	Poor	10,500	7,000
*8 B	70%	"	476,500	4,000

*Exposed to street dust.

An analysis of Tables III, IV and V showed comparative values of count and sanitary conditions. Table III showed samples purchased under market conditions. Only three of the samples were ground for the purchasers. Of the eleven samples (whose grinding history was not known) showing counts of 100,000 and over, five were bought near the end of the market where they were exposed to the dust of the street. Two were rated medium; three poor. Only one sample of less than 100,000 was found where meat was exposed in such

manner. Of the other six samples showing high counts, one 1 A, came from a covered counter; 4 C from a stall of fair sanitary conditions; and the others, 2 A, 4 A, 1 B, 3 A, from places rated poor. Of the six samples, 2 A, 4 A, 1 B, 24 C showed dextrose fermenting organisms to a count of over 1,000 per gram. All of these came from open pans or exposed counters.

Of the seven remaining samples of less than 100,000, five (10 C, 29 C, 31 C, 3 C, 33 C) showed no dextrose fermenters and the other two showed not more than 2,000. Six came from good sanitary surroundings and one from a stall of doubtful cleanliness. The three remaining samples were ground for the purchaser. All showed a count of approximately 100,000 and over. Sanitary conditions in these cases were good, with the exception of the fact that in two cases the meat was exposed to the street dust.

Of the 27 samples obtained from shops, 18 were ground or chopped while the purchaser waited and 9 were ready for sale. All but three of these showed dextrose fermenters of over 1,000. Of these, one (15 C) was cleaver-cut and showed 90% mold, and the other two (28 C, 18 C) were a poor quality of meat, as indicated by the proportion of fat and muscle

present (Section B 2). Only two of the ground samples, on first examination, showed over 100,000 bacteria per gram. One of these was block-cut; the other came from a very unsanitary shop.

The part the method of grinding played in total count was shown here. In only 3 of the 18 cases was the grinder thoroughly cleaned. In sample 13 C, where the sanitary conditions were good, the count was low, but 63% were dextrose fermenters. In the second case, 17 C, the grinder was carefully cleaned, but the meat was cut from a dirty piece exposed on a block. The count was comparatively high with low dextrose fermenters. The sample of the third case, 20 C, was composed of discolored scraps removed from the ice box before grinding. The count was likewise high but the fermenters were low. The significance of this variation in dextrose fermenters was by no means clear.

In 10 of the remaining 12 samples in which the grinder was not cleaned, the first small portion was discarded. In the two, 11 C and 12 C, all of the old grindings were put in. In spite of the fact that the sanitary conditions of the shops were in absolute contrast, the total counts, as well as the number of dextrose fermenters, were comparatively alike.

12 C and 26 C were cleaver-cut. 9 C, 16 C, 6 C, 14 C, and 19 C came from good sanitary surroundings and showed a total count varying from 6,000 to 34,000, and dextrose fermenters from 14% to 33 $\frac{1}{2}$ %; while 5 C, 20 C, 27 C, with poor sanitary conditions showed a range of 13,000 - 139,000 in total count, and in dextrose fermenters from .04% - 54%. The shop samples of which the grinding history was not known were all a poor quality of meat and came from shops of poor sanitary surroundings. In five, 8 B, 4 B, 5 B, 6 B, 7 C, the counts were over 100,000 and in four of these, 8 B, 4 B, 6 B, 7 C, there were a large number of fermenters. All of the latter came from shops which have the custom of opening the counters to the streets on market days.

2. A Comparison of Bacterial Counts and Organoleptic Tests in Shop and Market Samples

Comparing the results of Tables III to V, in Table VI it was noted that the market samples had a somewhat larger range of bacterial count than the shop samples: market 5,000 to 10,000,000 bacteria per gram; shop, 6,000 to 1,424,000 bacteria per gram. Only one of the 22 samples of the former fell below 10,000 bacteria per gram and four of the latter.

The market samples graded very good to good show comparatively the same bacterial count as those of the same group purchased in shops; while the counts in the samples graded medium to poor are decidedly higher in the market samples than in shop samples. The organoleptic test in neither shop nor market samples show any relation to total bacterial count. Dextrose fermenters were found in 12 of the 22 market samples. From these facts it is evident that there is no relation between the organoleptic test, total count, and the number of dextrose fermenters in any grade of meat. Most ground samples show a lower range in the bacterial count than samples not ground.

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3. Summary of All Samples Showing Relation
of Bacterial Count to Sanitary Shop Rate
and Organoleptic Test.

The 49 samples were graded as follows: 6, Very Good; 17, Good; 21, Medium to Fair; 5, Poor. The groups showed an overlapping in the sanitary rating of the shop. However, the limiting ratings of each group decreased in the same manner as the organoleptic tests (Table VII). This decrease was such that ratings of shops from which poor samples came were similar in some cases to the ratings of shops in which good samples were purchased.

This same relationship is seen in the total bacterial counts. The limiting counts gradually increased as the organoleptic test decreased, but the lower bacterial counts of those samples graded from Medium to Poor are included in the bacterial counts (Table VII) of those graded from Very Good to Good. No significance can be attached to the dextrose fermenting organisms present. The counts recorded for samples from Medium to Fair are included in those rated Good. The relation between the organoleptic tests, sanitary shop conditions, and bacterial count of all samples showed a decided overlapping in range.

TABLE VII

Summary of All Samples

	Sanitary Rating of Shop	Organoleptic Test	Range in Count Bacteria per grm.	Range in Dextrose Fermenter. per gram
	80%-95%	Very Good	6,000- 408,000	2,000 - 4,000
	55%-90%	Good	5,000- 300,000	2,000 -40,000
	30%-85%	Medium-Fair	20,000- 2,800,000	4,000 -26,000

The counts are lower than those of LeFevre 1917 (16), but his readings were four to five days after plating. These were read in 24 hours. They were smaller in some cases than those of Weinzirl and Newton (32) and in others they agreed. With Carey's (4) results of 24 hour readings at 37° they were comparable.

4. Seasonal Variations in Bacterial Count.

Weinzirl and Newton (32) found slightly lower counts in the winter months than the summer months. These samples were obtained in five different months. Nothing of significance was found in seasonal variation (Table VIII).

TABLE VIII
Seasonal Counts

Month Purchased	Range of Count
December	64,500 - 476,000
January	5,000 - 10,000,000
February	6,000 - 4,800,000
September	16,000 - 169,000
October	105,000 - 1,424,000

D. CHANGE OF BACTERIAL COUNT AND SPOILAGE

1. Organoleptic Changes in the Meat.

One hundred and sixty-two examinations were made on samples at two day intervals. Five samples were discarded at the end of two days; 28 at the end of four days; 12 at the end of six days; 2 at the end of eight days; 3 at the end of ten days; and one at the end of twelve days. The end point used for discarding the samples was such that the complete change of appearance would have kept them from human consumption. Not all changes as the samples spoiled were the same and they may be roughly divided as follows:

1- Those which produced an odor and were not moldy:

(a) Not discolored but produced strong odor.

(b) Discolored and produced foul odor.

(c) Slimy and produced foul odor.

1) Yellow color

2) Green color

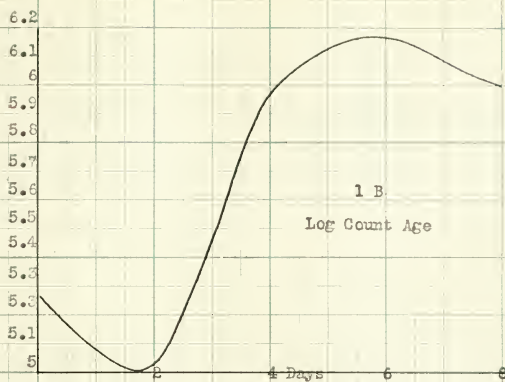
3) Black color

4) Slight change in color.

2- Those which were covered with a growth of mold:

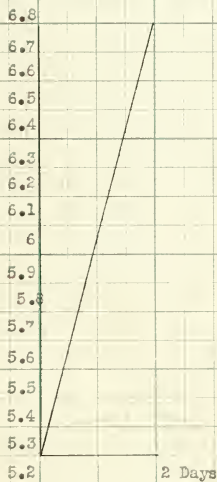
(a) Mold present, no change in color.

(b) Mold present with slight change in color.



1 B
Log Count Age

Fig. 1



2 A
Log Count Age

Fig. 2

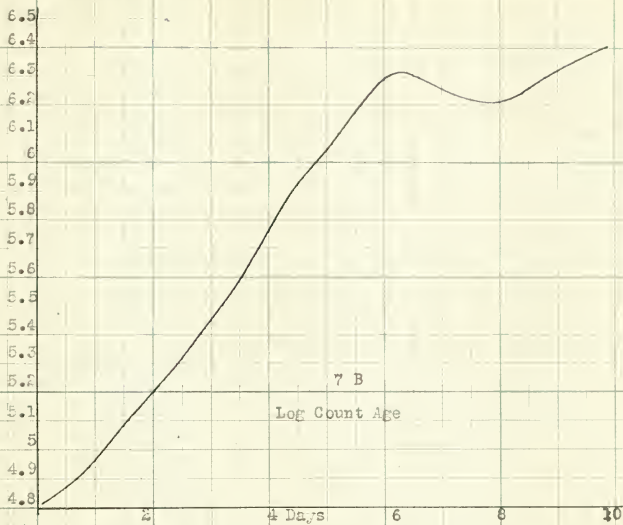


Fig. 3

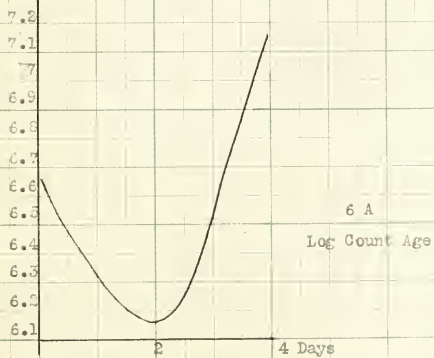
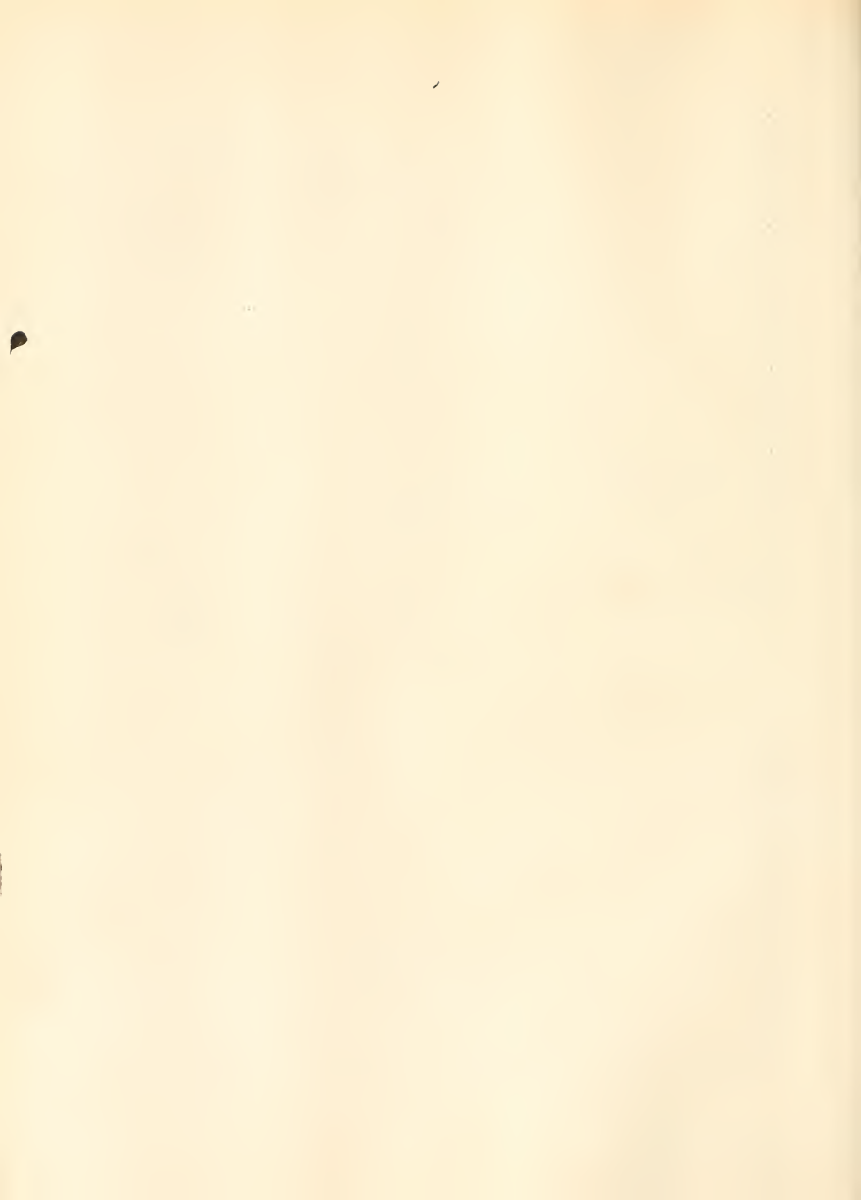


Fig. 4.



2. Change in Bacterial Count as the Sample Aged.

The change in count as the sample aged showed no relation to the end point reached. The rate at which the organisms increased showed no relation to the original count. Nine of the samples showed a decrease on the second day; nine a decrease in the final count; twenty-one a steady increase. The curve of the log count, plotted against increasing age of samples kept the longest before complete change took place, is indicated in Figure I. The other curves, Figures II, III, IV, showed different age curves of samples kept for a shorter time. These seemed to be sections of the longer curve. Whether Figure I represented the general trend of change in count of meat as it ages, and Figures II, III and IV indicate sections of that curve can only be determined by the examination of hundreds of samples whose age history is known. It was of interest to note that all samples which showed this decrease came from markets. No relation between this and the ratio of increase in count could be determined. Newton and Weinzirl (33) found Hamburger steak in cold storage showed a decrease after two weeks and then a marked increase.

The bacterial count of samples which were freshly ground increased for the most part with greater rapidity than those purchased already ground. This was easily explainable on the basis that grinding broke up the muscle tissue which offered a most favorable medium for growth. There was a steady increase in dextrose fermenters as the sample aged, with the exception of two cases. In one of the latter no dextrose fermenters were isolated. This increase showed no relation to the initial total count or initial count of dextrose fermenters.

The wide variation in the above results and the lack of the agreement between sanitary conditions, original organoleptic tests, and total count made the use of bacterial total count or count of dextrose fermenters a most unsatisfactory standard for meat analysis.

E. AEROBIC SPECIES PRESENT

Six hundred and forty-five forms were isolated from 15 samples. In 7 samples all organisms isolated were identified by their cultural reactions. In the other 8 samples those forms which could be identified as cocci or spore formers, microscopically, were recorded as such. All uncertain forms were identified

biochemically. This was done in order to determine if any form or group of organisms was so constantly present that it could be used as an indicator of the state of decomposition of meat.

The forms fell into the three morphological groups: (1) rods which were spore formers; (2) rods which were non-spore formers; (3) cocci. Of the 645 forms isolated 182 were cocci; 317 rods were non-spore formers; 146 rods, spore formers.

1. Spore Forming Rods.

Three spore bearers were isolated: *Bacillus albolactus*, *Bacillus subtilis*, *Bacillus vulgaris*, named in the order of their frequency.

2. Cocci.

Twelve cocci were isolated. Hunter (8,9) reports 16 from salmon, Carey (4), *Staph. aureus* twice from sausage. Eight cocci were named: *Micrococcus liquefaciens* (isolated by Tissier and Martelly (28) from meat; *Staph. candidus*; *Staph. candidans*; *Micrococcus cremoides*; *Staph. aurantiaca*; *Micrococcus roseus*; *Sarcina aurantiaca*; *Sarcina flavescens*. The last six were isolated by Riley (25) from air in Baltimore.

Hunter (19,11), Carey (4), and Tissier and Martelly (28) reported Streptococci but none were found here.

3. Chromogenic Forms.

The chromogenic organisms isolated were few in number. Besides the cocci mentioned above there were four rods: Pseudomonas fluorescences; two yolk colored ones (all of which were isolated by Hunter from salmon); and a brilliant red form.

Upon purification all the organisms were grown on gelatin, dextrose litmus agar, litmus milk, and plain broth, and the biochemical reactions were read on 1% dextrose, maltose, saccharose and mannitol broths. They were examined for their gram staining reaction.

4. Gram Negative Non-spore Bearing Rods.

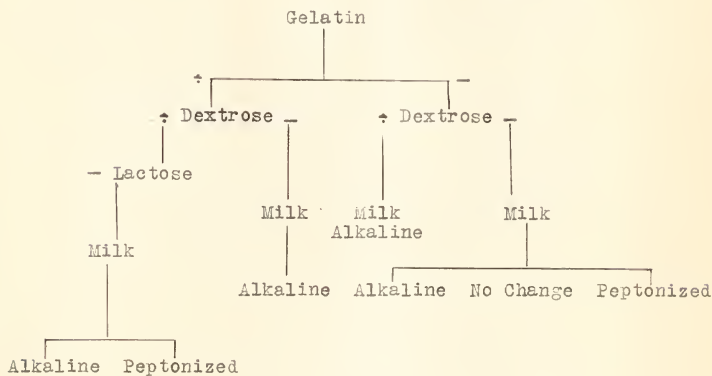
The small ^{gram} negative non-spore producing rods presented a rather different problem. There was constantly present a gas producing and non-gas producing group. Bacillus coli communis was isolated from 11 samples, Bacillus coli communior from 11, Bacillus proteus vulgaris from 5, Bacillus proteus mirabilis from 2, Bacillus cloacae from 2. Le Fevre, Tissier and Martelly, Hunter, and Carey

all reported *Bacillus coli*. The last three reported *Bacillus proteus vulgaris* and the last *Bacillus cloacae*. Three undetermined gas producing forms were isolated. One gave all the biochemical reactions of *Bacillus coli communior*, but the colony on agar was thin and wrinkled. From one sample, another was isolated which gave reactions of *Bacillus enteritidis*, and from two samples a third form which gave biochemical reactions of *Bacillus paratyphosus A*. Carey (4) isolated similar forms from sausage, but they would not agglutinate with the serums of *Paratyphosus A* and *Bacillus enteritidis*. For this reason he called them *Bacillus paracolon*. It seemed probable these were the same type of organism. They were not found in great frequency.

It is of advantage to summarize here the reactions of non-gas producing, non-spore bearing rods. There were 11 types. Practically all forms were isolated by Hunter 1922 (10) from salmon and sea water, by Conn 1917 (5) from soil, and by Jordan 1903 (14) from river water. Their distribution is evidently wide.

Jordan 1903 (14) divides the species into groups on the basis of their reaction on gelatin and milk. The species isolated in this study fall

into Jordan's Group IX, which liquefies gelatin and renders milk alkaline; Group XI, gelatin is not liquefied, milk alkaline; Group XII, gelatin not liquefied, milk not changed; Group XIII, chromogenic group. Their fermentation reactions on 1% dextrose and 1% lactose broth marked a distinct factor in their classification. They were found in great frequency when the numbers of gas producing rods were low. Their reactions may be summarized as follows:



5. Yeasts and Molds.

Yeasts were isolated in six cases. Hunter (8,9) reports yeasts in four cases of salmon decomposition and Carey (4) eight times in sausage. Molds were isolated in practically all cases. These belonged largely to the *Pencillium* and *Mucor* groups.

6. Correlation Between Bacterial Groups

Present in Samples.

The percentage of the different groups present was estimated. They divided themselves into the four logical divisions:

- (1) High percentage of spore formers--low
rods, low cocci
- (2) Low percentage of spore formers--low
rods, high cocci
- (3) Low percentage of spore formers--high
rods, low cocci
- (4) Approximately equal division of each.

TABLE IX

Correlation Between Groups Present in Samples

Samples	Percentage Spore Formers	Percentage Rods	Percentage Cocci
3 B	46%	29%	25%
3 A	45	0	55
7 B	37	21	42
4 B	36	26	38
1 A	33-1/3	0	66-2/3
5 A	25	43 $\frac{3}{4}$	33 $\frac{1}{4}$
2 B	13	6	81
2 A	11-1/3	55	33-2/3
1 B	11-2/3	55	33-1/3
7 A	10	65	25
5 B	10	30	60
8 B	7-1/3	26	66-2/3
6 A	5	83	12
6 B	4	46	50
4 A	0	86	14

There was constant balance between the percentage of non-spore forming rods and cocci present (Table IX). For example: Sample 2 B had 81% cocci and 6% rods; sample 6 A, 12% cocci, 83% rods; 3 B, 29% rods, 25% cocci. Since the three

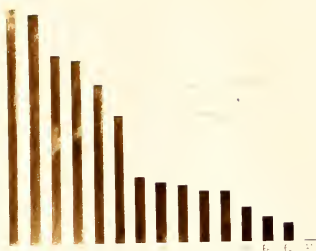


Fig. 5.

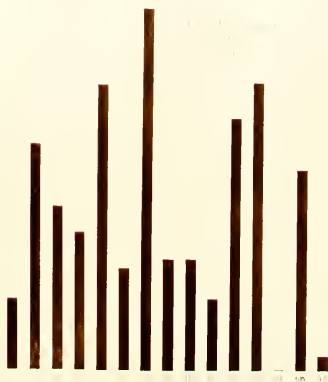


Fig. 6.

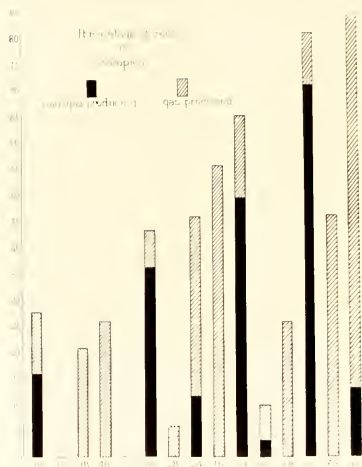


Fig. 7.

morphological groups--spore forming rods, non-spore forming rods, and cocci--were constantly present, the percentage of spore forming rods would determine the percentage of other species present. Therefore, these were used as a basis of comparison for the correlation indicated by figures V, VI, and VII and summarized in Table IX.

This held in all of the 48 examinations made as the samples aged. But the initial percentage of spore bearers, rods or cocci in no way seemed to influence the rate or ratio of increase.

7. Succession of Aerobic Species Present.

When the dominance of no group showed the state of decomposition, it was thought there might be a definite succession of aerobic species present.

The 15 samples were discarded as follows:

33-1/3%	2 days after purchase
40%	4 days after purchase
6-2/3%	6 days after purchase
13-1/3%	8 days after purchase
6-2/3%	10 days after purchase.

Of those destroyed at the end of two days (Table I) 6-2/3% showed no rods, 13-1/3% showed maximum development at time of purchase, 13-1/3%

showed maximum development at time of destruction. Of the 40% destroyed on the fourth day, 26-2/3% reached maximum rod development on the second day after purchase, 13-1/3% on the fourth day. Of the 6-2/3% destroyed on the sixth day all showed maximum development on the fourth day. Of the 13-1/3% destroyed, 6-2/3% reached maximum rod development on the day of purchase and 6-2/3% on the sixth day. All destroyed on the tenth day reached maximum rod development on the eighth day.

TABLE X.

Relation of Maximum Rod Production
to Day of Discarding Sample

Day Sample discarded	No Rods	Maximum Rod Production				
		Day purchased	Second day	Fourth day	Sixth day	Eighth day
2	*6-2/3%	*13-1/3%	13-1/3%			
4		0	26-2/3%	13-1/3%		
6		0	0	6-2/3%		
8		6-2/3%	0	0	6-2/3%	
10		0	0	0		6-2/3%

* Percentage of samples reaching maximum on day indicated above.

The cocci maximum (Table XI) of 26-2/3% of those destroyed on the second day was reached on the day of purchase; 6-2/3% on the day of destruction. Of

the four day group, 20% reached cocci maximum of growth on the first day, 6-2/3% on the second day, and 13-1/3% on the fourth day of storage in ice-box. In the six-day group, all reached cocci maximum on day purchased. In the eight day group 6-2/3% on date of purchase and 6-2/3% on the fourth day. Of the ten day group all reached cocci maximum two days after purchase.

TABLE XI

Relation Cocci Maximum to Day of Discarding Sample

Day discarded	Maximum Cocci Production		
	Day purchased	Second day	Fourth day
2nd	26-2/3%	6-2/3%	13-1/3%
4th	20%	6-2/3%	
6th	6-2/3%		
8th	6-2/3%	6-2/3%	
10th		6-2/3%	

From this it is seen that the maximum production of rods and cocci in spoilage is very variable. The outstanding facts are that in 50% the maximum in rods was reached two days before discarding, and 61% reached the cocci maximum on the day of purchase.

Less than 50% of the samples showed the maximum of spore formers on the day of destruction. In 66-2/3% of the samples the cocci maximum was reached before the rod maximum; in 6-1/3% it coincided with it, and in 26-2/3% it followed it. The maximum spore formation in 26-2/3% of the cases coincided with the cocci maximum. Therefore, there seems to be no definite succession in the growth of the aerobic forms.

In 33-1/3% of the samples the spore formers disappeared at the last examination and in 40% the cocci had disappeared. With the exception of one case, where no non-spore bearing rods were isolated, they were persistent to the end. In the 34 samples examined later for dextrose fermenters, the latter were found throughout the period of study of each sample.

8. Percentage of Species Which Liquefy Gelatin Present in Seven Samples

In the first seven samples in which a complete bacterial analysis was made, the percentage of gelatin liquefying organisms was determined. It was found in those which showed an end point of discoloration and strong odor, the percentage varied from 7 to 77%. In those which spoiled with a slimy, brown color and foul odor, there were from 20% to 54% present.

9. Summary of the Relation of Aerobic Species
to Sanitary Conditions and Type of
Spoilage in Meat.

TABLE XII.

Relation of Type Organism to Sanitary Conditions and Spoilage

Form Dominant	Number of Samples	Sanitary Rating of Shop	Organoleptic Test	Day first change noted	Day dis- carded	Type of Spoilage
Non-spore bearing rods	5	37%-70%	Good - Medium Fair - Poor	2-4-6	2-8	Slimy, moldy, odor foul. Brown
Cocci	4	32%-95%	Good - Medium Poor	2-4	4-6	All kinds*
Spore bearing rods	6 6	37%-95%	Good - Medium Fair - Poor	2-4-6	2-10	All kinds

* Section D, 1.

An analysis of Table XI shows that the type of aerobic organism present bears no relationship to the sanitary rating of shops, to the organoleptic tests, to the day when the first change in the sample was noted, or to the type of end change.

F. LACTOSE-DEXTROSE FERMENTATIONS.

Fermentation tubes, each of 1% dextrose and lactose broth, were used to determine the possible presence of gas producing organisms; $\frac{1}{2}$ cc. of the 1-20 dilution of the meat emulsion was added to the tubes. All samples showed gas in both media. Those which did not show it on the first examination showed it as the sample aged. In 15 samples the presence of *Bacillus coli* was confirmed, as well as other dextrose and lactose fermenters.

W. W. Browne 1917 (3) pointed out the fallacy of using the percent of gas found in fermentation tubes as an indicator of the amount of contamination in water analysis. In order to test this point, dextrose broth fermentation tubes and dextrose litmus agar were used. This was chosen in order to get an indication of the presence of non-lactose-fermenting forms. The results of 114 examinations, summarized in the Table XIII, showed no quantitative relation between percentage of fermentation and number of gas producing organisms present, confirming the observation of Browne.

TABLE XIII

Percentage of Fermenting Organisms and Percentage
of Gas Produced in 1% Dextrose

Number of Samples	Percentage of Gas Formed	Range of Count Dextrose Fermenters
11	75%	2,000 - 786,750
35	50%	0 - 800,000
32	25%	0 - 2,850,000
35	Below 25%	0 - 155,000

G. ANAEROBIC SPECIES PRESENT

1. Method Used in Isolating Organisms

Tissier and Martelly (28) reported three anaerobes in meat: *Diplococcus magnus anaerobius*, *Bacillus gracilis putidis*, *Bacillus putrificus coli*. Le Ferre reported one which he failed to identify. One hundred and fifty-two examinations were made for anaerobes on 44 samples, using the following technique. Two tubes each of deep litmus milk, 1% lactose, 1% dextrose broth and liver broth were inoculated with small portions of meat after the tubes had been boiled to expel the air. After inoculation they were heated in a water bath to 80° for ten minutes to kill the vegetative cells of non-spore bearing organisms present, cooled rapidly and incubated for from two to three days in a

McIntosh and Fildes (17) jar. One cc. of the contents of each tube was then removed by means of a sterile pipette and added to 1 cc. of sterile water and heated again to 80° for ten minutes. One cc. of this was inoculated into Rettger's (21) meat and egg medium and incubated in a McIntosh-Fildes jar. Unheated portions of the original cultures were used as checks on those which were heated before inoculation into the meat tubes and in all cases the same anaerobes were isolated.

From any tube showing signs of digestion, plates were made on 2% liver agar. These were plated from five to seven times in order to obtain pure cultures. They were then grown, for identification, on alkaline egg broth, egg cube, litmus milk, brain, gelatin, meat and egg media and 1% dextrose, maltose, saccharose, lactose, mannit, raffinose, and starch broths. Layering with sterile vaseline to obtain anaerobiosis was found very satisfactory to use in the identification of the organisms.

2. Descriptions of Anaerobic Species

Three hundred and forty-seven anaerobes were isolated; 102 showed no proteolytic action on meat and probably belong to Henry's (8) saccharolytic

group, as modified by Kahn; 115 showed strongly proteolytic action; and 130 were weakly proteolytic in varying degrees. The latter no doubt belong to Kahn's (15) medium group. One hundred and ten cultures which were identified represent six different types.

1) Saccharolytic Types

Twenty-seven cultures gave the reactions similar to Type I.

Type I

1. Morphology: A large bacillus occurring singly or in pairs. Distinct capsule in broth cultures as stained by His's stain. Spores, when found, oval in shape, larger than the bacillus and placed in a subterminal position.
2. Motility: Non-motile.
3. Staining Properties: Gram positive.
4. Cultural Reactions: At 37° C.

(1) Liver agar plates: Colonies white opaque to brownish white; oval, small to medium, and often surrounded by gas bubbles.

(2) Milk: Acid clots within eighteen hours, becomes colorless, loosely organized mass shot by gas bubbles.

(3) Broth:

- (a) Liver--Uniformly turbid, clearing within 36 hours. Gas production. Vaseline cap raised 2 cm.
 - (b) 1% Glucose. Turbid; violent gas production. Vaseline cap raised 4 to 5 cm. Acid* positive.
 - (c) 1% Saccharose. Turbid; violent gas production. Vaseline cap raised 4 to 5 cm. Acid positive.
 - (d) 1% Lactose. Turbid. Gas production. Vaseline cap raised 2 to 3 cm. Acid positive.
 - (e) 1% Raffinose. Turbid. Gas production. Vaseline cap raised 3 to 4 cm. Acid positive.
 - (f) 1% Mannit. Turbid. No gas produced. Acid negative.
 - (g) 1% Starch. Turbid. Gas production. Vaseline cap raised 4 cm. Acid positive.
- (4) Meat and Egg Medium: At the end of 24 hours the meat is bright red. Gas produced violently. Vaseline cap raised 4 cm. Liquid above the meat turbid. End of

*Acid test: Andrade indicator.

seven days meat volume is slightly reduced in size and liquid becomes clear, the meat particles remaining red and in no way decomposed.

(5) Solidified Egg Albumin Cube. No change in egg cube. Gas raises vaseline cap $1\frac{1}{2}$ to 2 cm. Broth turbid.

(6) Brain. Gas produced; vaseline cap raised 1 cm.

(7) Gelatin Stab. Gas produced; vaseline cap raised 1 to 2 cm. At end of 24 hours gelatin liquefied.

(8) Alkaline Egg Fluid(Robertson's, 24).

No change in media. Gas produced; vaseline cap raised 2 cm.

5. Animal Experimentation: Four cubic centimeters of a broth culture of the organism were inoculated into the ear vein of a rabbit weighing 1 kilogram, 500 grams. It was killed five minutes later and incubated at 37° for 24 hours. The rabbit was distended with gas. At autopsy the liver presented a typical foamy liver of *Bacillus welchii*. The organism giving the above cultural reaction was recovered from the liver and the heart's blood.

This organism agrees with the original description of *Bacillus welchii*, as given by Welch and Nuttall (34) and later amplified by Adamson (1), with the exception of the fact that instead of slight softening of gelatin, complete liquefaction took place. Weinberg and Seguin (31) report slow and incomplete liquefaction; Kahn (15), using pure line strain cultures, complete liquefaction.

Type II.

1. Morphology: Long, very slender organism.

Occurs singly. Spores are much wider than the bacillus and placed in a terminal position.

2. Motility: Motile.

3. Staining Properties: Gram positive.

4. Cultural Reactions:

(1) Liver agar: Colony white, round, and somewhat irregular.

(2) Milk: Solid clot, acid, later decolorized. Slight production of gas.

- (3) Broth:

(a) Liver. Turbid. Gas production; vaseline cap raised 2 cm.

(b) 1% Glucose. Turbid. Gas production; vaseline cap raised 2 cm. Acid positive.

- (c) 1% Saccharose: Turbid; gas production; cap raised 1 cm. Acid positive.
- (d) 1% Lactose. Turbid ; gas production; cap raised 1 cm. Acid positive.
- (e) 1% Raffinose. Turbid; gas production; cap raised 1 cm. Acid positive.
- (f) 1% Mannit. Turbid; gas production; cap raised 1 cm. Acid positive.
- (g) 1% Starch. Gas and acid negative.
- (h) 1% Maltose. Gas produced; vaseline cap raised 2 cm. Acid positive.
- (4) Meat and Egg Medium. Pink color. Slight production of gas.
- (5) Solidified Egg Albumin. Sediment at bottom of tube. Egg cube untouched.
- (6) Brain. Broth turbid on top of medium. No change in brain tissue.
- (7) Gelatin stab. No liquefaction of gelatin. Gas production. Vaseline cap raised 1 cm.
- (8) Alkaline Egg Broth. No change in medium.

The organism agrees with Henry's (8) *Bacillus tertius* in every way except in its action on alkaline egg broth. This reaction was similar to that described by Kahn (15) and Robertson (24). The bio-

chemical reactions disagreed with Kahn's (15) in that fermentation was found, as reported by Henry (8), in mannit and not in starch. This form was only isolated twice.

2) Medium Types.

Type III.

1. Morphology: Rather large, thick bacillus, occurring singly or in short chains. Spores large, about the same width as the bacillus. Located centrally or subterminally.
2. Motility: Feebly motile.
3. Staining Properties: Gram positive.
4. Cultural Reactions:
 - (1) Liver agar plates. Colonies white opaque; slightly irregular, surrounded by gas bubbles; odor foul.
 - (2) Milk. Clot regular and compact; small amount of gas. Clot and serum separate and the former settles to the bottom of tube and becomes soft. Serum red to amber color.
 - (3) Broth:
 - (a) Liver. Turbid; gas production; vaseline cap raised 2 cm.
 - (b) 1% Glucose. Turbid; gas production; vaseline cap raised 5 to 7 cm.Acid positive.

- (c) 1% Saccharose. Turbid; no gas or acid produced.
 - (d) 1% Lactose. Turbid; no gas or acid produced.
 - (e) 1% Raffinose. Turbid; slight gas and acid production.
 - (f) 1% Mannit. Turbid; no gas or acid produced.
 - (g) 1% Starch. Turbid; no gas or acid produced.
 - (h) 1% Maltose. Turbid; gas production; vaseline cap raised 1 to 3 cm.
- (4) Meat and Egg Medium: Meat at the end of 24 hours became a dark red. Gas production was slight. Vaseline cap raised 1 cm. After 36 to 48 hours the volume of the meat showed a decrease and there was deposited at the top a brownish jelly-like ring which increased in size as the culture aged. Black sediment in tube. Odor putrid.
- (5) Solidified Egg Albumin Cube:
Egg cube slightly ragged about the edge.
Broth turbid. Gas production; cap raised 2 cm.

(6) Brain: Medium darkened at the top. Gas production slight; vaseline cap raised $\frac{1}{2}$ cm.

(7) Gelatin Stab: Slow, feeble liquefaction.

(8) Alkaline Egg Fluid (Robertson):

Egg fluid thickened; later partial digestion takes place. Gas production; cap raised 1 cm.

This organism agrees with the description of Sacqueppe (26) for *Bacillus bellonesis*, except that it grew well in egg medium and showed partial digestion of egg broth. This reaction was more like that described by Weinberg and Sequin (31) and later amplified by Kahn (15), for *Bacillus bifermentans* (Tissier and Martelly). The milk reaction, however, is typical for *Bacillus bellonesis* and not for *Bacillus bifermentans*. This was the most abundant type present.

Type IV

1. Morphology: Short, plump bacillus, occurring singly and in pairs. Spores subterminal and not larger than the bacillus.
2. Motility: Feebly motile.
3. Staining Properties: Gram positive.

4. Cultural Reactions:

- (1) Liver agar plates. Colonies, oval, transparent; gas present around colonies.
- (2) Milk. Coagulated, acid, discolored in 24 hours; forms spongy mass.

(3) Broth:

- (a) Liver. Turbid; gas production; vaseline cap raised 2 cm.
 - (b) 1% Glucose. Turbid; gas production; vaseline cap raised 2 cm. Acid positive.
 - (c) 1% Saccharose. No gas or acid production.
 - (d) 1% Lactose. Turbid; gas production; vaseline cap raised 2 cm.; acid positive.
 - (e) 1% Raffinose. No gas or acid production.
 - (f) 1% Mannit. No gas or acid produced.
 - (g) 1% Starch. No gas or acid produced.
 - (h) 1% Maltose. Gas production; vaseline cap raised $1\frac{1}{2}$ cm.
- (4) Meat and Egg Medium. Meat brownish red. Liquid above medium at first turbid and then

clear. Gas produced; vaseline cap raised $1\frac{1}{2}$ cm. Slight digestion.

(5) Solidified Egg Albumin Cube. Gas produced; vaseline cap raised 2 cm. Broth turbid.

(6) Brain. Slight digestion in medium; gas produced; vaseline cap raised 1 cm.

(7) Gelatin Stab. Slow liquefaction.

(8) Alkaline Egg Fluid (Robertson's).

No change in medium.

The above mentioned organism agrees with Weinberg and Sequin's (30) description of *Bacillus aerofaecitides* with the exception of the fact that there was no attack on egg cube medium, and it differs from Kahn's (15) description of the same organism in that the milk clot softens.

3) Strongly Proteolytic Species.

Type V.

1. Morphology: Medium sized bacillus with rounded ends. Spores terminal and subterminal.
Spores wider than vegetative cell.
2. Motility: Actively motile.
3. Staining Properties: Gram positive.

4.Cultural Reactions:

- (1) Liver agar plates. Colony white opaque.
Irregular filament-like projections. Odor
of plate putrid.
- (2) Milk. Clotted; rapidly digested.
- (3) Broth:
 - (a) Liver. Turbid; putrid odor; gas
production; vaseline cap raised 5 cm.
 - (b) 1% Glucose. Turbid; putrid odor;
gas production, cap raised 3 cm.
Acid positive.
 - (c) 1% Saccharose. Turbid; no gas or
acid production.
 - (d) 1% Lactose. Turbid; putrid odor;
gas production; vaseline cap raised
1 cm. Acid positive.
 - (e) 1% Raffinose. Turbid; putrid odor;
gas production, vaseline cap raised
1 cm. Acid positive.
 - (f) 1% Mannit. Turbid; no gas or acid
production.
 - (g) 1% Starch. Turbid; no gas or acid
production.
 - (h) 1% Maltose. Turbid; gas production;
vaseline cap raised 2 cm. Odor putrid.
Acid positive.

- (4) Meat and Egg Medium: Meat darkened, digested, with the production of gas. Vaseline cap raised 3 cm. Residue of meat brownish red. Odor foul.
- (5) Solidified Egg Albumin Cube Broth. Egg cube digested in 24 hours. In 48 hours a slimy residue; gas production; vaseline cap raised $\frac{1}{2}$ cm. Odor foul.
- (6) Brain. Darkened; one half digested in 76 hours. Broth turbid; odor foul.
- (7) Gelatin Stab. Liquefied.
- (8) Alkaline Egg Fluid (Robertson). Medium clotted; clot rapidly digested; odor foul; gas production; vaseline cap raised 1 cm.

This organism agreed in detail with Metchnikoff's (18) description of *Bacillus sporogenes* and Kahn's (15) amplification of it; but differs from Weinberg and Sequin's *Bacillus sporogenes* and Koch's, as cited by Adamson (1), *Bacillus oedemates maligni*, as described by Jensen, by fermenting lactose.

Type VI.

Type VI showed the same cultural reactions as Type V but did not ferment lactose, and therefore these seem to be two different organisms. This

type agrees with the description of *Bacillus oedematis maligni*, Koch and Jensen, as cited by Adamson (1), and *Bacillus sporogenes* of Weinberg and Sequin (31), but differs from Kahn's (15) *Bacillus sporogenes* by not fermenting lactose. This organism was found in great abundance.

3. Relation of Anaerobic Forms to Types of Spoilage Changes

The strong proteolytic or medium forms were present in 28 samples. The final organoleptic change in such samples was similar--discolored, slimy appearance, with a foul odor. In all cases the first changes of odor and color were noted within two days. This period would probably have been shortened at room temperature.

In the other ten samples, which showed weak proteolytic or saccharolytic forms, no change was observed before four to six days. This was a slight to strong odor, slight change in color, and often the appearance of mold.

4. Summary of the Relation of Anaerobic Species to Shop Conditions, Organoleptic Test and Bacterial Count

Table XIV indicates the relation between the shop conditions, the original organoleptic test, the

FOLD OUT

Sac.	Day first change	Day final change
	2	8
2+	2	6
	2	6
2-	2	6
	2	8
	2	6
	2	4
2	2	8
2-	2	4
2-	2	4
	2	8
2-	2	4
2-	2	6
	2	4
	2	6
2-	2	8
2	2	4
	2	6
	2	4

FOLD OUT

Sac.	Day first change	Day final change
------	------------------------	------------------------

2-	2	6
----	---	---

	2	4
--	---	---

	2	4
--	---	---

	2	4
--	---	---

	2	4
--	---	---

	2	4
--	---	---

2-	2	4
----	---	---

	2	4
--	---	---

	2	4
--	---	---

	4	4
--	---	---

2	4	4
---	---	---

2-	4	6
----	---	---

2	4	4
---	---	---

2+	4	4
----	---	---

	4	4
--	---	---

2-	6	6
----	---	---

2	6	6
---	---	---

2	6	8
---	---	---

2	4	4
---	---	---

Red lettering indi.
of shop, and bact.

presence of anaerobes, bacterial count and spoilage changes. In the cases where the strongest proteolytic forms (Table XIV, 30 C to 26 C) were isolated, the sanitary shop conditions ranged from 30% to 95%, and the original organoleptic tests show five samples rated Very Good, seven Good, ten Medium, four Fair, and two Poor. The bacterial count of six samples are below 10,000 and six above 100,000, while the other twelve range from 11,000 to 93,000. The number of dextrose fermenting organisms vary from 0 to 23,000. All but three of the samples were shop samples, and 12, or 50% were ground while the purchaser waited.

With the exception of four samples, saccharolytic anaerobes were isolated from all the samples but only persisted in eleven cases, while the strongly and medially proteolytic species were found in all examinations. The constant features of this group are the similarity of the end point reached in physical appearance of the samples as the result of spoilage--namely, foul odor, slimy, discolored appearance (Section G,3)--and the fact that the first changes in the physical appearance came within two days after purchase. There was no relation between the original organoleptic test or bacterial count and

the features cited above. The presence of these species in no way indicated the time in which complete change in the sample would take place. In the other group, 31 C to 32 C (Table XIV), where weakly proteolytic and saccharolytic species were dominant and strongly proteolytic bacteria absent, the sanitary rating of shops varies from 30% to 80%, and one sample is rated organoleptically Very Good, five Good, three Medium, one Fair, and two Poor. None of the samples fell below 10,000 in bacterial count and 6, or 50%, were above 100,000. The dextrose fermenting organisms ranged from 0 to 40,000. In no case were strongly proteolytic species isolated. Six of the twelve samples were market samples and three only were ground for the purchaser.

The final changes in physical appearance is not so marked as in the other group, but again is very similar; namely, strong odor, slight to medium change in color, and the appearance in some cases of a growth of mold (Section C 3). In all cases the first changes in appearance came later than in the first group-- four to six days. These bore no relationship to the initial bacterial count, the original organoleptic test, the time of complete spoilage or the sanitary rating of the shop. Therefore, the type of anaerobic species seems to indicate the type of change in physical appearance which takes place

in the sample and the time the first changes will occur.

H. SUMMARY AND DISCUSSION.

From the observations mentioned in this paper it may be seen that the possibility of using the aerobic bacterial count and aerobic bacteria as a standard for meat analysis has been eliminated. The bacterial count of market samples shows a wider range than those of shop samples, and the count of freshly ground samples is lower in general than those not freshly ground. The bacterial count of freshly ground samples is lower in general than those not freshly ground. The bacterial count of all samples shows much variation in the relation to the sanitary conditions and the organoleptic test, and there is no relation between the initial bacterial count and counts as the meat ages. The presence of dextrose fermenting species in no way indicates the age or the state of decomposition of the meat.

Of the 645 organisms isolated, 328 were of undoubted environmental origin (cocci and spore forming rods); 317 were of fecal or doubtful origin. Although the non-spore bearing rods were the most constant species present, no succession of aerobic species was found as the sample aged. There was no relation between the gelatin liquefying aerobes and

spoilage in the sample. The infrequency with which the members of the Paratyphoid and Enteridites groups were found made the possibility of food poisoning under existing conditions from that source slight.

Three types of anaerobes were isolated: proteolytic, medially proteolytic, and saccharolytic, and the presence of each group is indicated by a definite type of spoilage. Although each type of spoilage represents a special type of anaerobe, there is no relation between the anaerobe present, the original organoleptic test, the sanitary conditions, and the aerobic count.

The presence of proteolytic anaerobes seems to indicate a definite way by which the keeping qualities of meat can be predicted. The technique already described for indicating their presence can be easily adopted for laboratory use. The McIntosh-Fildes jar can be dispensed with and anaerobiosis can be successfully obtained by layering with vaseline. The meat tube, according to Holman (9), remains anaerobic

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without any further treatment. The length of time for reading results could thus be shortened. This, however, does not indicate the possible source of the contamination of the meat; 87 $\frac{1}{2}$ % of the samples which showed these organisms were purchased in shops as in contrast to open market stalls, while 50% of those which did not show proteolytic anaerobes were purchased in markets. The actual exposure to dust from the street is greater in the market stalls than in the shops, but if a shop became infected with an organism it would probably be harder to eliminate than in the larger open market stall. If the source of the proteolytic types V and VI are fecal, it is an entirely different problem than if they are of environmental origin. The contamination may then either come from improper slaughtering or later from unsanitary handling.

The question of utmost importance to the sanitarian is that of possible toxic properties found in the meat produced by these organisms during spoilage. This is a biochemical problem.

I. CONCLUSIONS

An analysis of the observations made in this study justify the following conclusions.

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1. Total aerobic bacterial count or count of dextrose fermenters is unreliable as a standard for meat analysis.

2. There is no relation between initial bacterial count and bacterial counts as the sample aged.

3. The presence of an aerobic species or group cannot be taken as an index to the freshness of the meat.

4. There was a definite correlation between the percentage of organisms present at each examination.

5. There was no relation between the percentage of proteolytic aerobic species and the type of spoilage.

6. Three types of anaerobes were present: strongly proteolytic, medially proteolytic, saccharolytic.

7. The presence of strongly proteolytic forms produced a definite type of spoilage in meat: foul odor, brown color, slimy appearance. The first indication of change of this type took place within two days at ice-box temperature.

8. Those samples with weak proteolytic or saccharolytic forms present spoiled with little change in color, a strong but not foul odor, and in many cases had the appearance of mold. The first indication of change in these cases occurred within four to six days.

9. There was no relation between the presence of anaerobes, the original organoleptic test, sanitary conditions, or aerobic count.

10. The presence of proteolytic anaerobes indicates the probability of early spoilage.

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